Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity?

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SUMMARY

The major histocompatibility complex (MHC) is an immunologically important group of genes that appears to be under natural as well as sexual selection. Several hypotheses suggest that certain MHC-allele combinations (usually heterozygous ones) are superior under selective pressure by pathogens. This could influence mate choice in a way that preferences function to create MHC-heterozygous offspring, or that they function to create specific allele combinations that are beneficial under the current environmental conditions through their complementary or epistatic effects. To test these hypotheses, we asked 121 men and women to score the odours of six T-shirts, worn by two women and four men. Their scorings of pleasantness correlated negatively with the degree of MHC similarity between smeller and T-shirt-wearer in men and women who were not using the contraceptive pill (but not in Pill-users). Depending on the T-shirt-wearer, the amount of variance in the scorings of odour pleasantness that was explained by the degree of MHC similarity (r^2) varied between nearly 0 and 23%. There was no apparent effect of gender in this correlation: the highest r^2 was actually reached with one of the male odours sniffed by male smellers. Men and women who were reminded of their own mate/ex-mate when sniffing a T-shirt had significantly fewer MHC-alleles in common with this T-shirt-wearer than expected by chance. This suggests that the MHC or linked genes influence human mate choice. We found no significant effect when we tested for an influence of the MHC on odour preferences after the degree of similarity between T-shirt-wearer and smeller was statistically controlled for. This suggests that in our study populations the MHC influences body odour preferences mainly, if not exclusively, by the degree of similarity or dissimilarity. The observed preferences would increase heterozygosity in the progeny. They do not seem to aim for more specific MHC combinations.

1. INTRODUCTION

Products of the major histocompatibility complex (MHC) play a crucial role in immune recognition because they present antigens on cell surfaces to patrolling T lymphocytes (Klein 1986). The extraordinary variability of MHC genes is therefore thought to be maintained by some sort of parasite-driven balancing selection (reviewed in Potts & Wakeland 1993; Brown & Eklund 1994; Hedrick 1994). In human populations heterozygosity of the MHC is more frequent than expected by chance (Hedrick & Thomson 1983). This seems to result from natural selection as well as sexual selection: MHC-heterozygotes may, on average, have a selective advantage under pathogen pressure (e.g. Doherty & Zinkernagel 1975; Hughes & Nei 1988; see discussion in Brown 1997). Sexual selection, on the other hand, includes mate choice and maternal selection before, during and after fertilization (Potts & Wakeland 1993; Wedekind 1994*a*). In mice, some of these possible selection levels have already been shown to be important and mostly, but not always, lead to an excess of MHC-heterozygous offspring (Yamazaki *et al*. 1976, 1983*b*; Egid & Brown 1989; Potts *et al*. 1991; Wedekind *et al*. 1996). Odours have been shown to play an important role in mate choice (Yamazaki *et al*. 1979; Egid & Brown 1989). Humans are also able to discriminate the odours of congenic mice strains that (ideally) differ only in their MHC (Gilbert *et al*. 1986), and rats seem to be able to recognize human MHC-types from urine odours (Ferstl *et al*. 1992). A sensitivity for MHC-correlated odours within our own species could also be observed in a study with university students by Wedekind *et al*. (1995). They found that women's perception of male body odours correlates with their respective MHC. Here again, odour-based mate choice would lead to an increased rate of MHC-heterozygous offspring, but only in women who were not using the contraceptive pill. Recently, Ober *et al*. (1997) partly confirmed these findings in a study on American Hutterites: the MHC-types of 411 couples were more often different from each other than would be expected if matings were random (in

Table 1. *Characteristics of the six T-shirt-wearers and average scorings of their odours by the 121 smellers*

T-shirt-wearer	associations to tobacco smoke or perfume	MHC-types	average scoring of		gender discrimination
			intensity (range)	pleasantness (range)	
M1	<1%	A2, A3; B7, B7; DR15, DR15	5.7 (0.6–10)	4.4 (0–10)	t=0.98$^{n.s.}$
M2	0	A2, A3; B7, B14; DR7, DR15	7.2 (2–10)	3.5 (0–10)	t=9.80**
M3	0	A9, A11; B7, B35; DR1, DR4	6.1 (0.3–10)	4.5 (0–10)	t=0.47$^{n.s.}$
M4	21%	A9, A11; B5, B35; DR15, DR4	6.2 (0–10)	6.3 (1–10)	t= − 3.17* (wrong sex)
F1	0	A1, A3; B8, B18; DR3, DR4	4.2 (0–9)	5.4 (0–10)	t=3.10*
F2	<1%	A1, A3; B8, B7; DR3, DR15	4.6 (0.6–10)	5.3 (0–10)	t=4.16**

M1–M4 = male T-shirt-wearers; F1, F2 = female T-shirt-wearers.
*p < 0.01; **p < 0.001; two-tailed.

their calculation of the null expectancies they had controlled for non-random mating with respect to colony lineage and with respect to kinship).

In our previous experiment (Wedekind *et al*. 1995) we had only asked women to sniff male odours. However, in our human study population, axillary organs important for odour production occur in both sexes. Therefore, a first aim of the present study was to test for gender effects in the perception of body odours. We also aimed to test whether we were able to reproduce the main findings of Wedekind *et al*. (1995) with a different experimental design and new pairs of odour-producers and smellers.

There are two groups of possible evolutionary explanations for MHC-dependent mate preferences. First, because of its extraordinarily high polymorphism, the MHC may serve as a marker for the degree of kinship between two individuals (Brown 1983; Uyenoyama 1988; Potts & Wakeland 1993; Brown & Eklund 1994; Potts *et al*. 1994). Avoiding MHC-similar individuals as mates would reduce the likelyhood of inbreeding and the negative fitness consequences associated with it (Potts *et al*. 1994; Charlesworth & Charlesworth 1987). The second group of hypotheses suggests that certain MHC combinations are superior under selection by pathogens (reviewed in Potts & Wakeland 1993; Hedrick 1994; Potts *et al*. 1994; Wedekind 1994*a*). Such parasite-driven sexual selection could simply aim to create heterozygosity on an immunologically important gene complex, i.e. the combinations of any different alleles on loci of the MHC (Yamazaki *et al*. 1976; Potts & Wakeland 1993; Hedrick 1994; Brown 1997). Alternatively, this selection could aim to create specific allele combinations that are beneficial under the current parasite pressure through the complementary or epistatic effect of the different MHC-alleles (Wedekind 1994*a*,*b*; Wedekind *et al*. 1996). Most of these beneficial allele combinations may be heterozygous ones, but this for itself need not reveal a direct causal relationship. Epistatic effects of at least two human MHC-alleles to a schistosomal antigen have been found by Hirayama *et al*. (1987), and the strong linkage disequilibria observed between many alleles of the MHC (Bender 1991) could be explained by long-term epistatic fitness effects (Maynard Smith 1989). However, beneficial epistatic effects need not be long term; they may also be continously changing during host–parasite coevolution. Because of the possibility that some allele combinations are more beneficial than others under a given selection pressure, a well-tuned condition-dependent selection mechanism could result in a non-trivial fitness advantage. Such a conditional choice would take into account the present pathogen pressure and would promote allele combinations that ensure the highest fitness returns. This requires physiological mechanisms that have not been demonstrated so far. Nevertheless, the present study was designed to test whether individual odour preferences would lead to such specific allele combinations in the progeny.

2. METHODS

A total of 121 male and female students or lab assistants participated in this study as 'smellers' (58 women, average age 26.0 (s.d. = 3.9); 63 men, average age 25.7 (s.d. = 2.9); age not significantly different between the sexes: $t = 0.49$, n.s.). All participants were informed about the aims of the study and gave their consent after the theoretical background and possible consequences of the study had been explained. They were all connected to Bern University and all spoke the Swiss-German dialect without any foreign accent (to define our study populations). We typed them for their HLA-A, -B, -DR (methods described in Wedekind *et al*. 1995).

Two women and four men (age between 21 and 25 years, armpits not shaved, and with MHC-alleles that are, on average, quite common in the study population, see table 1) were asked to wear a T-shirt (100% untreated cotton) during a Sunday and Monday night, to keep the T-shirt in an open plastic bag in between, and to live as much as possible odour-neutral during these two days (detailed rules in Wedekind *et al*. 1995). This procedure was repeated on five consecutive weekends during June and the first week of July, with the same six T-shirt-wearers, but with new T-shirts each time.

On each following Tuesday some of the smellers scored the odours of the six T-shirts. The smellers were naive about the degree of their MHC-similarity to the T-shirt-wearers and about the gender of the T-shirt-wearers. The T-shirts were provided in numbered, glazed cardboard boxes laid out with plastic foil (PVDC) (the numbers on the boxes were changed between different test weeks). A triangular hole allowed the smellers to sniff the contents. On top of the cardboard boxes was an additional layer of foil, which was changed after every test run. This way, the triangular hole on top of the box was covered and the foil had to be cut to open the triangle hole before every test run. This was done

Table 2. *Median (range) of the six Pearson's correlation coefficients r between the age of the evaluators and their perception of odour intensity, and between the scores of odour pleasantness and scores of odour intensity, for the different groups of smellers*

(Within each comparison, the correlation coefficients do not differ significantly between the groups of smellers; Wilcoxon, p always >0.05.)

group of smellers	Pearson's r
age (smeller) versus scores for intensity	
men	−0.45* (−0.66; 0.09)
women not on the Pill	−0.33* (−0.57; 0.05)
women on the Pill	−0.43* (−0.60; −0.02)
scores for pleasantness versus intensity	
men	−0.22* (−0.52; −0.10)
women not on the Pill	−0.26* (−0.32; −0.01)
women on the Pill	−0.18 (−0.37; 0.11)

*Wilcoxon, test against 0, $n=6$, $p<0.05$, two-tailed.

with a scalpel in the presence of the test subject to demonstrate that the top plastic foil was fresh and hygienic. Alone in a room, every smeller was allowed about 20 minutes to score the odours of the T-shirts on a continuous scale for intensity (range 0–10) and pleasantness (range 0–10, 5 = neutral), and to guess the gender of the T-shirt-wearers (range 0–10, 0 = surely female, 5 = ambiguous, 10 = surely male). A marked box with an unworn T-shirt was provided to allow the smellers to control for the experimental setup's own odour. They were also asked to write down memory associations, especially to relatives, present or former mates, and whether the odour reminded them of perfume or tobacco smoke. We were unaware whether some smellers had strong homosexual preferences, i.e. we did not ask anyone for their sexual preference.

Whenever possible, female smellers scored the odours in the second week after the beginning of menstruation (Pill-users: 11.8 d (s.d. = 6.4); non-Pill-users: 12.8 d (s.d. = 5.3); $t=1.22$, n.s.), as women appear to be most odour-sensitive during that time (Doty *et al*. 1981). Because oral contraceptives are a potentially confounding factor in this type of study (Wedekind *et al*. 1995), we asked each woman whether she was taking the Pill at the time of the experiments. This was the case in 26 of the women (45%).

None of the T-shirt-wearers had any relatives who participated in the study as smellers, and the 121 smellers had 117 different family names, representing a group of mostly, if not completely, unrelated individuals (one of two twin brothers was excluded from the analysis by tossing a coin).

Of the 121 smellers, 81 had not sniffed T-shirts during our previous study (Wedekind *et al*. 1995), and two of the six T-shirt-wearers had already acted as T-shirt-wearers in the previous study. This resulted in 18 combinations of odours and smellers that had already occurred in the first study (i.e. only 2.5% of all combinations of the present study). For these 18 cases we could not find a significant consistency in the scorings for pleasantness or intensity between the two studies (scorings for pleasantness: $r=-0.06$, n.s.; scorings for intensity: $r=0.16$, n.s.), and none of these smellers had the same memory associations when sniffing the same odour in the first and in the second study. These non-significant correlations cannot be interpreted as non-repeatability of our previous findings because 18 pair-combinations are only 6.1% of all pair-combinations of the previous study, i.e. a small subsample with low statistical power for an analysis of repeatability. For these reasons we regard the analyses of the present study as sufficiently independent from the previous one.

If predictions were directed, we used directed tests (Rice & Gaines 1994); parametric statistics were used only when sample sizes were large ($n>25$) and when data plots had indicated that the assumptions of parametric statistics were not violated; for smaller samples sizes non-parametric statistics were used. We used SYSTAT (Systat 1992) for the data analyses.

3. RESULTS

(*a*) *Intensity and pleasantness of odours, gender discrimination*

One T-shirt-wearer may not have followed the rules for the experiment correctly, as his odour reminded many smellers of tobacco smoke and/or perfume (table 1). However, the other five T-shirt-wearers seemed to have followed the rules. Table 1 also lists the MHC-types of the T-shirt-wearers, their average scores for intensity and pleasantness, and how well the smellers guessed their gender.

(i) *Intensity*

The six odours were scored differently with respect to intensity (repeated measure ANOVA, $F=32.7$, d.f. = 5, $p\ll0.001$), and the smellers were quite similar in their relative scorings of intensity of the six odours (figure 1, left bar). However, they differed in their absolute scorings, which was partly explained by their age, i.e. older smellers described the odours as less intense than younger ones (table 2). This seemed to be independent of gender or whether or not the women were on the Pill (table 2). There were also no other gender or Pill effects on the scorings of odour intensity (comparison between male and female smellers: ANOVA, $F=1.47$, d.f. = 1; n.s.; comparison between Pill-users and non-Pill-users: ANOVA, $F=0.003$, d.f. = 1; n.s.).

(ii) *Pleasantness*

Overall, the six odours were scored differently with respect to pleasantness (repeated measure ANOVA, $F=18.1$, d.f. = 5, $p\ll0.001$). The two women and the man whose odour often reminded the smellers of tobacco smoke and perfume tended to be scored as more pleasant (table 1), and the more intense an odour was, the more unpleasant it was scored by the smellers (table 2). In spite of these correlations and the finding that the relative scorings for odour intensity are similar between different smellers (see above), the smellers differed very much in their relative scorings for odour pleasantness (figure 1, right bar). The strong trend into negative correlations in figure 1 indicates already strong individual differences in the perception of body odour pleasantness. These differences cannot be explained with the age of the smellers (analysed separately for men, women on the Pill and women not on the Pill: Wilcoxon, $n=6$, p always >0.60, two-tailed), and they are also not explained by sex or Pill effects (comparison between male and female smellers: ANOVA, $F=2.05$, d.f. = 1; n.s.; comparison between Pill-users and non-Pill-users:

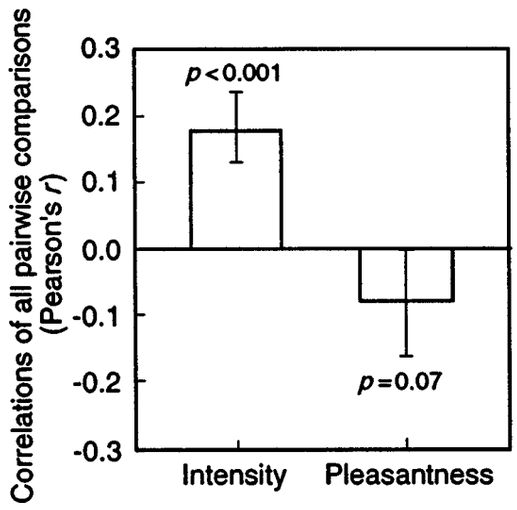

Figure 1. Pearson's correlation coefficients r of all possible comparisons of two individual odours out of the six ($=15$ pairwise comparisons, i.e. 15 rs) of the smellers' scorings for intensity of an odour (left side) and for its pleasantness (right side). The figure shows the medians and quartiles. The rs do not differ significantly between men, women not on the Pill and women on the Pill (Wilcoxon two-sample test, p always >0.05). The p-values in the figure stem from one-sample Wilcoxon tests (test against 0, two-tailed).

ANOVA, $F=1.54$, d.f. $=$ l; n.s.), but they are partly explained by the MHC (see below).

(iii) *Gender discrimination*

Nobody correctly guessed the gender of all six T-shirt-wearers. However, most could not decide in at least one case (average number of estimations per smeller 4.8, range 0–6; only 37% of all smellers gave an estimation of the gender of all T-shirt-wearers). The smellers tended to describe more pleasant odours as female and more unpleasant ones as male, apparently regardless of whether the T-shirt-wearer was actually male or female (all smellers pooled per T-shirt-wearer: $-0.21>r>-0.47$, p always <0.02). The men made this mistake more often than the women (Wilcoxon two sample test, $p=0.03$, two-tailed). The Pill did not seem to have an effect here (Wilcoxon two sample test, $p=0.92$, two-tailed). Only three of the six T-shirt-wearers could on average be correctly sexed (table 1). Female smellers were better than males in this respect (ANOVA, $F=14.7$, d.f. $=1$, $p<0.001$), and again the Pill did not seem to play a role here (ANOVA, $F<0.001$, d.f. $=1$, $p=0.998$).

(*b*) *Odour perception in relation to the MHC*

(i) *Memory associations*

In 45 cases an odour reminded a smeller of one of his/her relatives (mostly brothers, sisters, parents and self). If these memory associations were influenced by the MHC, one can expect on average more shared MHC-alleles in these smellers and the respective T-shirt-wearer. This did not appear to be the case (figure 2*a*, top). However, in 28 cases an odour reminded a smeller of his/her mate or former mate (14 times in men and 14 times in women). If these memory associations were influenced by the MHC, one can expect, on average, less shared MHC-alleles in these smellers and the respective T-shirt-wearer (Wedekind *et al*. 1995). This turned out to be the case, confirming that the MHC correlates with actual mate choice in our study population (figure 2*a*, bottom). Men and women did not differ significantly in the correlation between the MHC and memory associations to their mates (Fisher exact test, $p=1.0$). Six men and two women with memory associations to their mates were wrong in their gender discrimination and were all of the same gender as the respective T-shirt-wearer. This had no detectable influence on the correlation to the MHC (Fisher exact test, $p=1.0$).

(ii) *Pleasantness versus degree of MHC similarity between smeller and T-shirt-wearer*

For each odour we calculated the correlation between the score of pleasantness and the degree of MHC similarity between smeller and T-shirt-wearer, analysed separately for men, women on the Pill and women not on the pill. These correlations are summarized in figure 2*b*. Men and women who were not on the Pill tended to prefer odours of MHC-dissimilar T-shirt-wearers (figure 2*b*). Women on the Pill showed, on average, opposite but non-significant correlations between preference and MHC similarity (figure 2*b*). The amount of variance in the scorings of an odour's pleasantness that is explained in this study by the degree of similarity of HLA-A, -B and -DR alleles between T-shirt-wearers and smellers (r^2) varied between nearly 0 and 22.6% (highest r^2: Ml; male smellers), 14.7% (M4; female smellers not on the Pill), 16.6% (Ml; male and female smellers not on the pill pooled), and 13.5% (Ml; female smellers on the pill). The median r^2s per six T-shirts for every category of smellers can be deduced from figure 2*b*, and lie between 2 and 12‰. However, the r^2s of the six T-shirt-wearers differ significantly from each other in at least some categories of smellers (test for heterogeneity among six r^2s; male smellers: $\chi^2=12.2$, d.f. $=5$, $p<0.05$; female smellers not on the Pill: $\chi^2=4.5$, n.s.; male and female smellers not on the Pill pooled: $\chi^2=12.2$, $p<0.05$; female smellers on the Pill: $\chi^2=7.9$, n.s.). This indicates that the different odours varied in the degree to which they revealed the MHC of the respective T-shirt-wearer.

(iii) *Testing for preferences for specific MHC combination (when degree of MHC similarity is controlled for)*

For each odour we grouped the smellers for same sex, whether Pill-users or not, and for same number of MHC-alleles shared with the respective T-shirt-wearer. Only groups of at least five smellers were used. This resulted in 9–11 groups of smellers per T-shirt-wearer with on average 11.5 smellers per group. Within each of these groups we calculated each smeller's Spearman's correlation coefficient (r_s) between the number of MHC-alleles shared with each other smeller in the group and the similarity in the scoring of pleasantness

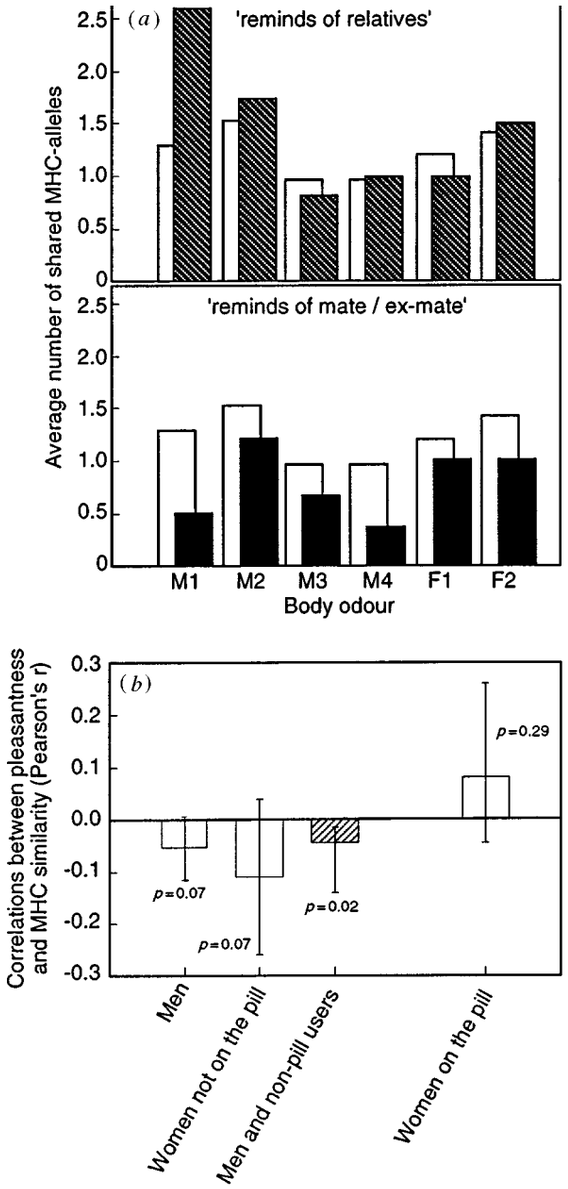

Figure 2. Correlation between the MHC and odour perception. (*a*) Average number of MHC-alleles shared between smeller and a T-shirt-wearer when the odour reminds the smeller of a relative (above, hatched boxes), or of his/her present or former mate (below, solid boxes) in comparison with the average number of MHC-alleles shared between all smellers and a T-shirt-wearer (open boxes). M1–M4 are male T-shirt-wearers, F1 and F2 are females. In 45 cases an odour reminded a smeller of a relative, but in only 22 of these cases (49%) did the smeller share more MHC-alleles with the respective T-shirt-wearer than expected by chance, and in only four of the six odours did the average number of shared alleles lie above the null expectancy (Wilcoxon, $p = 0.38$, directed). However, in 28 cases a T-shirt's odour reminded a smeller of his/her mate or former mate (14 male and 14 female smellers). In 20 of these cases (71%) the smeller shared less MHC-alleles with the T-shirt-wearer than expected by chance (Binominaltest, $p = 0.02$, directed), and in all six odours the average number of shared alleles was below the null expectancy (Wilcoxon, $p = 0.02$, directed). The sexes did not differ significantly in these MHC-correlated memory associations (see text). Ten of 14 memory associations in the lower graph were by women who were sure that they had not taken the contraceptive pill when they chose the particular mate they were reminded of. (*b*) Pearson's correlation coefficients r (medians and quartiles) between scores of pleasantness and the degree of MHC-similarity, i.e. the number of shared MHC-alleles between smeller and T-shirt-wearer (six rs each, i.e. for each T-shirt-wearer one correlation per category of smellers). The correlation coefficients are similar for men and women not on the Pill (Wilcoxon two-sample test, $p = 0.92$). The p-values in the figure stem from one-sample Wilcoxon tests ($n = 6$, directed according to the prediction from Wedekind *et al.* (1995)).

with each other smeller (i.e. the absolute difference in scores of pleasantness for the given odour). To get rid of pseudoreplication (within a group, the r_ss per smeller are not fully independent from each other) we used the average Spearman's r_s per group of smellers for further analyses. For each T-shirt-wearer we tested the average r_ss of the groups of smellers against zero. This allows us to examine whether smellers with similar MHC have similar odour preferences if we control for the degree of MHC similarity between smeller and T-shirt-wearer. In figure 3*a* the average correlation coefficients per group of smellers enter as independent data points. If the MHC influences the perception of odour pleasantness independently of the degree of MHC similarity between smeller and T-shirt-wearer, we expect, on average, negative r_ss. This does not seem to be the case: the group means are never significantly negative (figure 3*a*, in three of the six comparisions the group means are even slightly positive).

(iv) *Allele-specific analysis*

To test in a different way whether the MHC influences the perception of odour pleasantness independently of the degree of MHC similarity between smeller and T-shirt-wearer, we used the following procedure.

For each odour we split the smellers (except Pill-users) into those who scored the odour as unpleasant (scores of pleasantness < 5) and those who scored the odour as neutral or pleasant (scores of pleasantness $\geqslant 5$). As male and non-Pill-using female smellers have not shown any significant differences in their MHC-correlated odour perception (see previous analyses and figure 2), we pooled them to reach maximal statistical power for this analysis ($n_{\text{total}} = 95$). Then we counted, in both groups, the number of smellers who possess a given MHC-allele. For each of the six odours we performed this procedure for the three most common HLA-A, -B and DR-alleles in our study population that were not found in the respective T-shirt-wearer's own MHC. These numbers were compared to a null expectancy (derived from the total number of smellers in each group). None of the MHC-alleles analysed in this way showed a significant correlation to body odour preference (figure 3*b*).

To illustrate the analyses in an example: of 16 smellers who carried the allele HLA-A19, two scored the odour of T-shirt-wearer F1 as unpleasant, and 14

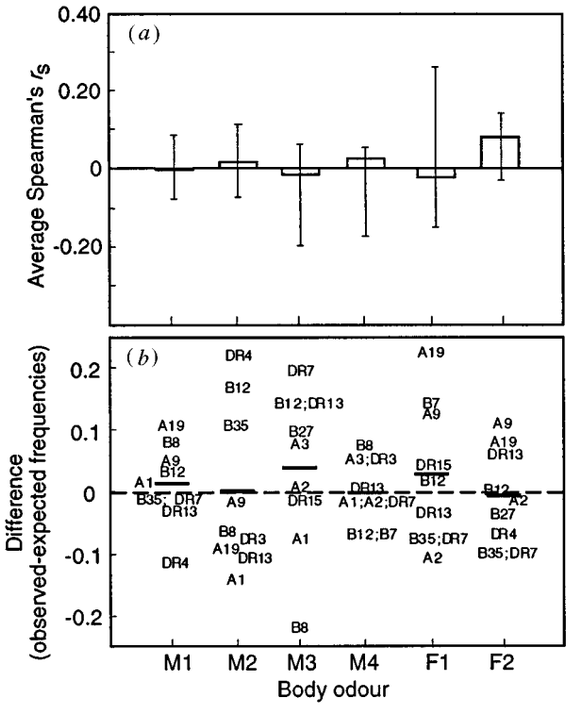

Figure 3. Test for MHC-dependent odour preferences when the degree of similarity between smeller and T-shirt-wearer is controlled for. (*a*) For each odour (M1–M4, F1 and F2) the smellers were grouped by the categories men, Pill-users and non-Pill users, and by the number of MHC-alleles shared with the T-shirt-wearer. For each smeller within each of these groups we calculated the Spearman's correlation coefficient (r_s) between number of shared MHC-alleles with all other smellers and the similarity in the scoring of pleasantness with all other smellers (i.e. the absolute difference in scores of pleasantness for the given odour). Only groups of at least five smellers for which five r_ss could be calculated were used (average group size = 11.5 smellers, s.d. = 6.2). In this way we got 9–11 groups of smellers for the six T-shirt-wearers. The average Spearman correlation coefficients enter the figure as independent data points. The figure shows medians and quartiles per T-shirt-wearer. In no case are the group means significantly smaller than 0 (Wilcoxon, p always >0.30, directed). The variance in these group means per T-shirt-wearer is to a large extent explained by the different group sizes (correlation between group size and absolute difference of the group mean to 0: $r = -0.52$, $p \ll 0.001$, directed). (*b*) Relative frequency of smellers who scored an odour as neutral or pleasant and possessed a given HLA-A, -B or DR-allele. Women on the Pill were excluded from this analysis. The figure shows the differences between the observed frequencies and the null-expectancy for each T-shirt-wearer, and for the three HLA-A, -B or DR-alleles that were most common in the study population but not found in the T-shirt-wearer's own HLA-type (to control for the degree of similarity between smeller and T-shirt-wearer, see text). None of these frequencies deviates significantly from the null-expectancy (after Bonferroni correction for multiple comparisons). The variance in the observed frequencies is partly explained by the different number of smellers with a given MHC-allele (correlation between group size and the absolute difference of the observed frequencies to the null-expectancy: $r = -0.246$, $n = 54$, $p < 0.05$, directed). The average differences between observed and expected frequencies (horizontal lines) are in no case different from 0 (Wilcoxon, p always >0.40, two-tailed).

smellers scored it as neutral or pleasant. The observed frequency is therefore 0.875 (14/16) and the expected frequency from the null hypothesis is 0.642 (61/95); the difference of 0.233 enters figure 3*b* (comparison between expected and observed frequencies in this case: $\tilde{\chi} = 1.94$, $n = 16$, $p > 0.05$ before Bonferroni correction for multiple comparisions).

Both analyses summarized in figure 3 suggest that the MHC influences body odour preferences in our study population only by the degree of similarity between smeller and T-shirt-wearer. When we control for this effect we cannot find a statistically significant influence by the MHC on the perception of body odours.

4. DISCUSSION

The MHC-correlated memory associations collected in this study are in accordance with our previous study on human body odour perception (Wedekind *et al.* 1995) and indicate again that the MHC or linked genes influence mate choice in our Bernish study population, in spite of the common use of soaps, deodorants and perfumes. Moreover, the finding that the perceived pleasantness of the body odours tended to correlate negatively with the degree of similarity on the MHC between T-shirt-wearers and smellers is further support for the main conclusion of our previous study, and is in accordance with findings in mice (see §1). In Pill-using women the correlation between odour pleasantness and the degree of similarity on the MHC tended to be again reversed. We speculated previously (Wedekind *et al.* 1995) that women on the Pill may, in some respect, xbehave like pregnant women, and maybe pregnant women have these opposite preferences because their odour preferences serve other aims (as it is the case in mice, see Manning *et al.* 1992). This hypothesis still needs to be tested, because Pill-using women could differ in several respects to non-Pill-using women.

In studies like this, with humans as subjects, we can only conclude that the MHC loci or linked genes influence body odour production and perception. However, the MHC is very likely to be the most important correlate, as Yamazaki *et al.* (1983*a*) found in mice that even differences as slight as a single gene mutation on the MHC can be recognized by odours. Furthermore, studies on outbred mice by Potts *et al.* (1991) and Yamazaki *et al.* (1994) suggest that the discrimination of MHC-odour types is not appreciably affected or impaired by other loci of the genome that are in one way or another a source of body odours. In our study, up to 23% of the variance in the perception of odour pleasantness could be explained by the MHC, but the six odours differed very much in this respect.

Body odour preferences in humans appear to be context-dependent. The likely influence of the contraceptive pill (observed in Wedekind *et al.* 1995; indicated in the present study) already suggests that odour preferences are also condition-dependent. Analogously, the subjective perception of many other odours is known to depend on one's physiological state or one's memory associations, e.g. odours of food that made one sick will be disliked afterwards. When sniffing body odours, it

may be possible that a subject's personal history influences his/her subjective perception (as it does in mice, see Yamazaki *et al*. 1988). This could potentially explain some of the variance in our data. The observation that a woman's preferences for body odours seem to depend on her hormonal status also fits into the general finding that MHC-correlated sexual selection normally depends on conditional factors (Yamazaki *et al*. 1988; Wedekind *et al*. 1996).

All the effects found in our previous study on humans (Wedekind *et al*. 1995) may appear to be slightly weaker in the present study, in which the sample size was even larger. This could be explained by the different design of the two studies. In our previous study we presented odours of the most extreme MHC similarity or dissimilarity that could be found for a given smeller among 44 T-shirt-wearers. Moreover, the preference scorings of three odours each were summarized to one value that entered the statistics. In this way we got rid of some true random error that we were not interested in and consequently achieved more statistical power to test whether or not there is a correlation between MHC and body odours. In the present study, all smellers sniffed on the same six odours, which were, for most of the smellers, of more or less average degree of MHC similarity, i.e. the variance between the six odours in the degree of similarity to the smeller's MHC was much lower than in the first study. Furthermore, scorings of odour pleasantness were not summarized for statistical analyses. This design is weaker for detecting a preference for unequal MHC-types, but it allowed us to estimate the amount of variance in odour pleasantness explained by the MHC (which turned out to be, on average, very low, although in some cases it was quite high). Furthermore, the current study design allowed us to test whether individual odour preferences can lead to specific combinations of MHC-alleles in the progeny (see §1). We could not find any indication for such a complex preference mechanism. This suggests that the MHC influences human body odour preferences only by the degree of similarity between smeller and T-shirt-wearer, resulting more often in heterozygous combinations of no further specificity. However, a preference for specific allele combinations is expected to be most beneficial only as a conditional response to current parasite pressure (see §1). To reach a safer conclusion in this respect, our study should be repeated with a more narrowly defined population that suffers from a higher pathogen pressure. It could be that our study population was 'too healthy' to find such specific preferences.

The findings of the present and our previous study do not allow for a discrimination between the two major groups of evolutionary hypotheses (which are not mutually exclusive), i.e. (i) the MHC serves as a marker for kinship to avoid inbreeding and (ii) parasite-driven sexual selection acts on the MHC itself because of its fundamental importance for the immune system. Only an effect of the MHC on odour preference when the degree of MHC similarity between T-shirt-wearer and smeller was statistically controlled for would not have been explainable by the inbreeding avoidance hypothesis and would have provided strong support in favour of the parasite-hypothesis.

To achieve a discrimination between these two hypotheses has been tried in mice, and data by Potts *et al.* (1994) actually suggest that inbreeding avoidance may be the most important function of MHC-based mate preferences in this rodent. Especially in small populations, MHC-based odour cues may allow for good estimates of the degree of kinship and may therefore be very valuable in avoiding the detrimental effects of inbreeding depression (Brown 1983; Charlesworth & Charlesworth 1987; Potts *et al*. 1994). Both hypotheses predict that preferences lead to an excess of heterozygotes in the progeny (see, for example, discussion in Brown & Eklund 1994; Potts *et al*. 1994; Brown 1997). As in our study the T-shirt-wearers were not related to any of the smellers, the observed MHC-correlated odour preferences can clearly be used in situations not involving relatives. This is in accordance with analogous findings in mice (see discussion in Potts *et al*. 1994).

Koelega & Köster (1974) concluded from a series of experiments that women are more sensitive to several distinct odours than men. Accordingly, we observed that women were better able to discriminate the gender of the T-shirt-wearers than men. This confirms previous findings (Hold & Schleidt 1977). However, we found no significant sex difference in the correlation between MHC and odour perception (if men were compared to non-Pill-using women only). The strongest correlation between MHC and odour pleasantness was even observed in one of the male odours sniffed by male smellers. This suggests that the MHC-correlated perception of odour pleasantness does not depend very much on whether the smeller and the T-shirt-wearer are of the same or of different sex.

The physiological link between the MHC and body odours in human and other mammals has not yet been sufficiently demonstrated. The human skin retains two types of glands apart from sweat glands, which secrete a watery fluid for the purpose of evaporative cooling (Stoddart 1991). On the one hand we have sebaceous glands located all over the body, which secrete an odourless oily liquid that is broken down by bacteria into volatile molecules, mostly fatty acids (Stoddart 1991). Another type of gland, the apocrine glands, are located mainly in the axillae but also on other parts of the body, which characteristically bear patches of springy hairs (Stoddart 1991). According to Stoddart (p. 58) apocrine glands may be '. . . the major source of scent with which a healthy human body is endowed'. This seems to be combined with bacterial activity, as one of the highest densities of bacteria on the surface of human skin are found on the axilla (Jackman 1982). These bacteria could be representative of organisms indigenous to human skin, as the composition of the bacterial flora in the axillae can quite remarkably differ between subjects (review in Jackman 1982). This could allow for a very indirect association between MHC and body odours (Howard 1977). As an alternative but non-exclusive hypothesis on the production of MHC-correlated odours, it could be that apocrine secretions contain molecules that reveal the allelic specificity of the MHC and that become volatile during bacterial decay (e.g. in analogy to the hypothesis proposed by Roser *et al.* (1991)).

Two different organs are known to mediate olfactory sensory perception in humans: the main olfactory epithelium (Engen 1982) and the vomeronasal organ (e.g. Wysocki 1989). Although olfactory receptor genes are located on several human chromosomes (Ben-Arie *et al*. 1994; Fan *et al*. 1995), at least some of them, which are expressed and polymorphic, are located telomeric of the HLA-A gene within only about 1000 kbp (Fan *et al*. 1995; A. Ziegler and co-workers, unpublished data). This close link between MHC and some olfactory receptor genes may partly be responsible for the MHC-correlated odour perception found in this study and our previous study.

Whatever the mechanism for production and perception of MHC-correlated body odours, the fact that the MHC or linked genes correlated to human body odour production, body odour preferences and actual mate choice in already two different test series, demonstrates that no one smells good to everybody, it depends on who is sniffing on whom, and it is correlated to their respective MHC.

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